Journal of Microbiological Methods 99 (2014) 71–80

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

A new ultrasonic high-throughput instrument for rapid DNA release from microorganisms

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ARTICLE INFO

Article history:
Received 27 November 2013
Received in revised form 4 February 2014
Accepted 6 February 2014
Available online 16 February 2014

Keywords:
Sonication
Microorganisms
Lysis
Universal
High throughput
PCR

ABSTRACT

Rapid detection methods for microbiological contamination are requested by many industries that respond to public health concerns. The control laboratories are replacing traditional culturing methods with faster assays based on nucleic acid amplification technologies, such as real-time PCR. However, an optimal nucleic acid sample preparation method is critical for the sensitivity and specificity of such tools.

A high-throughput automated external ultrasonic device was developed for rapid lysing of microorganisms. Based on Ct values obtained from real-time PCR, there was efficient DNA release from the 16 microorganisms tested, including Gram positive and negative bacteria, bacterial spores, yeasts and spores of molds. Linearity of the lysis method was also demonstrated for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Candida albicans* and the spores of *Aspergillus brasiliensis*, with correlation coefficients (r^2) between 0.90 and 0.98. After sonication, PCR analysis of the lysates revealed earlier Ct values (0.83 to 7.49) for *S. aureus*, *P. aeruginosa* and *S. enterica* compared to the bead-beating method of lysis. These results demonstrated more efficient DNA release from bacteria with the ultrasonication system. Nevertheless, for fungi, the Ct values were 0.94 to 1.61 later for sonication than for bead beating.

This study demonstrates that 4 min of sonication with this new automated high-throughput instrument allows for the efficient lysis of a large range of microorganisms.

1. Introduction

The rapid detection and analysis of microorganisms has become more and more essential for applications ranging from food safety and public health to environmental monitoring and biological weapons defense (Jimenez, 2001; de Boer and Beumer, 1999; Rao et al., 2010). The traditional methods for the detection of potential pathogens rely on culturing and plating of the bacteria, followed by species identification. These methods are time consuming, taking three to five days for the evaluation of total counts and up to seven days for specific pathogenic bacterial detection.

Quality control and research laboratories are increasingly willing to use rapid detection tools. Recent advances in this field have considerably improved speed and sensitivity (Jimenez et al., 2000; Wang et al., 2007). Most of these assays are based on nucleic acid amplification technologies. Indeed, methods such as real-time polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP) or transcription-reverse transcription concerted reaction (TRC) afford the shortest time to results, the best sensitivity and the highest specificity required for the detection of harmful microorganisms (McKillip and Drake, 2004; Mori and Notomi, 2009; Tanaka et al., 2010). However, various parameters may affect the sensitivity of real-time PCR assays, and an optimal nucleic acid extraction method is critical (Dauphin et al., 2009).

None of the current methods on the market allow for rapid, universal, high-throughput and reproducible lysis of microorganisms. A variety of chemical agents, such as lysozyme, proteinase K and detergents have been used to achieve cell lysis, but these reagents require time-consuming sample preparation in several steps before PCR analysis. Physical methods are also commonly used to extract nucleic acids from cells, such as bead-beating, French press, and heat or osmotic shock. Bead-beating presents many advantages due to its ability to quickly lyse a large number of samples simultaneously with good reproducibility. However, the size and type of beads used (e.g., glass, silica, ceramic) differ with the samples and microorganisms. Moreover, the beads must be removed from the sample carefully in order to not interfere with nucleic acid purification and detection steps (Rantakokko-Jalava and Jalava, 2002). However, none of the other disruption methods is as useful for fast, reproducible, universal and high-throughput DNA release.

The main hurdle that lysis methods must overcome is the high diversity of cell wall structures in microorganisms. Gram-positive bacteria are far more resistant to lysis than Gram-negative bacteria because of the rigid peptidoglycan structure in their cell wall, which is organized as a multilayered network (Mahalanabis et al., 2009).

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http://dx.doi.org/10.1016/j.mimet.2014.02.004
0167-7012/

Likewise, spores and fungal cells are strongly resistant to lysis due to their particular structure and often require a combination of techniques to prepare them for analysis (Karakousis et al., 2006; Ceuppens et al., 2010).

The purpose of this article is to analyze the performance of a sonication instrument developed for a quick, simple, reproducible, automated and high-throughput lysis of all types of microorganisms that may be present in a sample. Ultrasound technology was chosen for its ability to disrupt any cellular structure (Gerde et al., 2012; Chaiyarit and Thongboonkerd, 2009; Zhang et al., 2007), its ease of use and its easy incorporation into a sample-to-result instrument based on nucleic acid detection. Cell lysis and DNA release were evaluated using real-time PCR on a broad list of microorganisms with different cell wall organization (Gram positive and Gram negative bacteria), morphology (rods or cocci), and physiological structure (spores), as well as difficult-to-lyse cells such as fungi. The influence of the growth phase on lysis efficiency and the linearity of DNA extraction were also evaluated. A simultaneous comparison with bead-beating, a well-established cell lysis method, was also undertaken.

2. Material & methods

2.1. Microorganism cultivation

Except for the spores, all microorganisms were obtained from the American Type Culture Collection (ATCC), the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) or the National Collection of Type Cultures (NCTC) and were stocked in calibrated cryotubes at −80 °C after growing in broth. The following strains were used: *Enterococcus faecalis* ATCC 19433, *Staphylococcus aureus* ATCC 6539, *Staphylococcus epidermidis* ATCC 12228, *Listeria monocytogenes* ATCC 19116, *Streptococcus agalactiae* DSM 2134, *Propionibacterium acnes* ATCC 6919, *Clostridium sporogenes* ATCC 19404, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enterica* subsp. *enterica* NCTC 6676, *Escherichia coli* ATCC 25922, *Zygosaccharomyces bailii* DSM 70492, *Candida albicans* DSM 1386, *Cryptococcus neoformans* ATCC 14113, and *Saccharomyces cerevisiae* ATCC 7754. Calibrated dehydrated pellets of *Aspergillus brasiliensis* spores ATCC 16404 were purchased from Microbiologics (Epower ref. 0392E6) and calibrated suspensions of *Geobacillus stearothermophilus* spores ATCC 7953 were purchased from Amilabo (ref. 751045).

To evaluate sonication as a lysis method, the microorganisms (except spores) were first plated on the recommended culture media after thawing the cryotube at room temperature. Then, optical density (OD) and colony forming units (CFUs) were correlated for each microorganism (Table 1).

2.2. Design of the sonication instrument

Ultrasonic treatment was conducted with a custom-made 24-tube sonication instrument (Felden et al., 2014) (Fig. 1). The ultrasonic horn is a rectangular piece of aluminum with a footprint of approximately 76 mm by 120 mm. The horn contains a 4 by 6 grid of equally spaced wells. Each well has a specific shape that mimics the bottom of a conical round bottom tube (e.g., a 1.5-ml Eppendorf tube). Therefore, the energy transfer between the ultrasonic probe and the sample contained in the tube is maximized, allowing lysis without plunging the sonotrode in the samples. This design eliminates the risk of contamination between samples and the need to clean the instrument after each run. The sample tubes are kept vertically and an equal force is applied to the top of each tube using a system of pushrods with prestressed springs. A 750 W power board drives the probe using a piezoelectric converter (lead zirconate titanate crystals). The energy output is set to the maximal power (750 W) at a frequency of 20 kHz, which is the natural frequency of the horn. At this frequency mode, the amplitude of vibration is uniform for each well. Because the force applied to each tube and the amplitude of vibration are the same for each tube, the energy transmitted is the same to each position. The ultrasounds are applied in a continuous manner (duty cycle of 100%) with a ten second ramp-up phase to reach the power set point. The increase in temperature in the tubes is proportional over time, with a ramp-up of approximately 30 °C per min (Fig. 2).

Table 1
Culture conditions and the relationship of OD measurement/CFU concentration.

Microorganisms	OD measurement	Concentration (CFU/ml)	Culture media	Temperature of incubation
Enterococcus faecalis ATCC 19433	0.552	$6.8 \cdot 10^8$	TSA	32.5 °C
Staphylococcus aureus ATCC 6539	0.408	$4.4 \cdot 10^8$	TSA	32.5 °C
Staphylococcus epidermidis ATCC 12228	0.576	$1.6 \cdot 10^8$	TSA	32.5 °C
Streptococcus agalactiae DSM 2134	0.447	$2.2 \cdot 10^8$	COS	32.5 °C
Listeria monocytogenes ATCC 19116	0.538	$8.4 \cdot 10^8$	TSA	32.5 °C
Escherichia coli ATCC 25922	0.519	$5.3 \cdot 10^8$	TSA	32.5 °C
Pseudomonas aeruginosa ATCC 9027	0.482	$6.1 \cdot 10^8$	TSA	32.5 °C
Salmonella enterica subsp. *enterica* NCTC 6676	0.602	$7.7 \cdot 10^8$	TSA	32.5 °C
Propionibacterium acnes ATCC 6919	0.173	$1.1 \cdot 10^8$	COS	32.5 °C
Clostridium sporogenes ATCC 19404	0.430	$4 \cdot 10^7$	COS	32.5 °C
Zygosaccharomyces bailii DSM 70492	0.517	$2.3 \cdot 10^6$	SDA	25 °C
Saccharomyces cerevisiae ATCC 7754	0.532	$4 \cdot 10^6$	SDA	25 °C
Candida albicans DSM 1386	0.584	$7.9 \cdot 10^6$	SDA	25 °C
Cryptococcus neoformans ATCC 14113	0.483	$3.8 \cdot 10^6$	SDA	25 °C